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14. ABSTRACT

Hormesis describes a phenomenon where low doses of toxic substances or radiation stimulate responses that might counteract the harmful effects of a subsequent high level of stress. We hypothesized that low doses of radiation received through mammography could be beneficial for patients. We therefore treated human breast epithelial cells with X-rays at low doses, high doses, or at low doses followed by high doses. We then scored cells for the effects of these various doses on the number of DNA damage 53BP1 foci and on the ability to trigger cell senescence. Based on the results we have obtained so far, there was no significant change in the amount of DNA damage foci generated nor in the induction of senescence when cells were pre-treated with low doses of ionizing radiation and subsequently challenged by a high dose of ionizing radiation. Moreover, there was no effect on chromatin modifications in mammary epithelial cells, whereas low doses of radiation appeared to have some effects in human fibroblasts. In conclusion, our data suggest that low doses of radiation are not protective, nor detrimental, in mammary epithelial cells, whereas in other cell types, such as fibroblasts, these low doses of radiation appear to be protective.

15. SUBJECT TERMS
hormesis; radioadaptive response; mammography; breast cancer; mammary epithelial cells; fibroblasts; X-rays; senescence; DNA damage foci

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INTRODUCTION

Hormesis describes a phenomenon where low doses of toxic substances or radiation stimulate responses that might counteract the harmful effects of a subsequent high level of stress. It was suggested that low doses of radiation could induce a protective response against DNA damage and therefore cancer. The radioadaptive response is thought to result from positive feedback stimulation of cellular defense or repair pathways. Therefore, hormesis implies that, depending on the dose, radiation might be "friend" or "foe". We hypothesized that low doses of radiation received through mammography could be beneficial for patients and that there was a possibility for new methods of breast cancer prevention, particularly in subjects having a high risk of developing breast cancer, regarding the use of low doses of ionizing radiation. We therefore started to test the hypothesis that, in mammary epithelial cells, low doses of radiation protect cells from DNA damage and/or loss of cell function (such as cell senescence) caused by higher doses of stress. We also planned to determine whether these responses differ depending on the cell type (epithelial versus fibroblast).

BODY

First, we treated human breast epithelial cells with X-rays at low doses, high doses, or at low doses followed by high doses. We then scored cells for the effects of these various doses on the number of DNA damage foci (using immunostaining and an anti-53BP1 antibody) and on the ability to trigger cell senescence (using the SA-β-galactosidase staining).

METHODS

Cell culture: Human mammary epithelial cells (184-A1) were cultured using Lonza mammary epithelium basal medium (MEBM) with bicarbonate buffering and supplemented with Clonetics bullet pack (consisting of EGF, BME, insulin, and hydrocortisone).

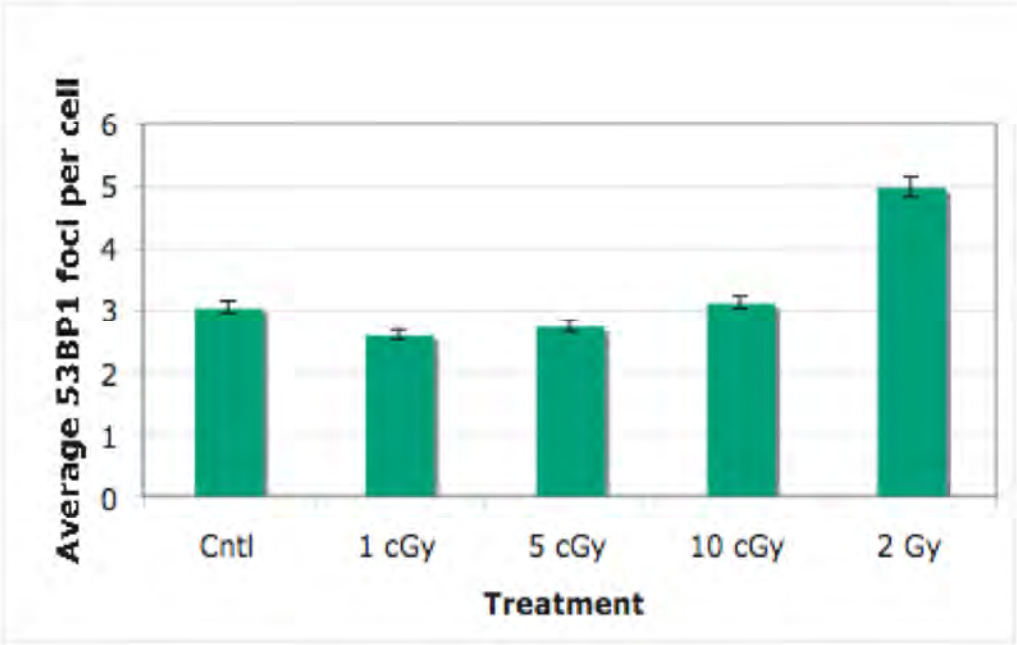
Ionizing radiation: Cells were plated and then irradiated the next day with high doses of 2 Gy at a dose rate of 150 Rad/min, and low doses 1 cGy and 5-10 cGy at dose rates of 4.6 Rad/min and 10.8 Rad/min respectively. Intervals between low and high doses ranged from 4-48 h and collection after high dose varied from 0 h-10 d.

Immunofluorescence for DNA damage foci: Cells were seeded at 50,000 cells/well in 4-well chamber slides. They were fixed with 4% paraformaldehyde, washed with PBS, and incubated with the primary antibody anti-53BP1 (rabbit from Bethyl 1:1000), and then with a secondary antibody. Cells were quantified using the Cell Profiler software.

SA-β-galactosidase staining for senescent cells: We used the staining kit from Biovision with cells grown in 6-well plates or 35 mm dishes. Senescent cells positive for β-galactosidase activity (blue in color) were counted using a microscope and imaged.

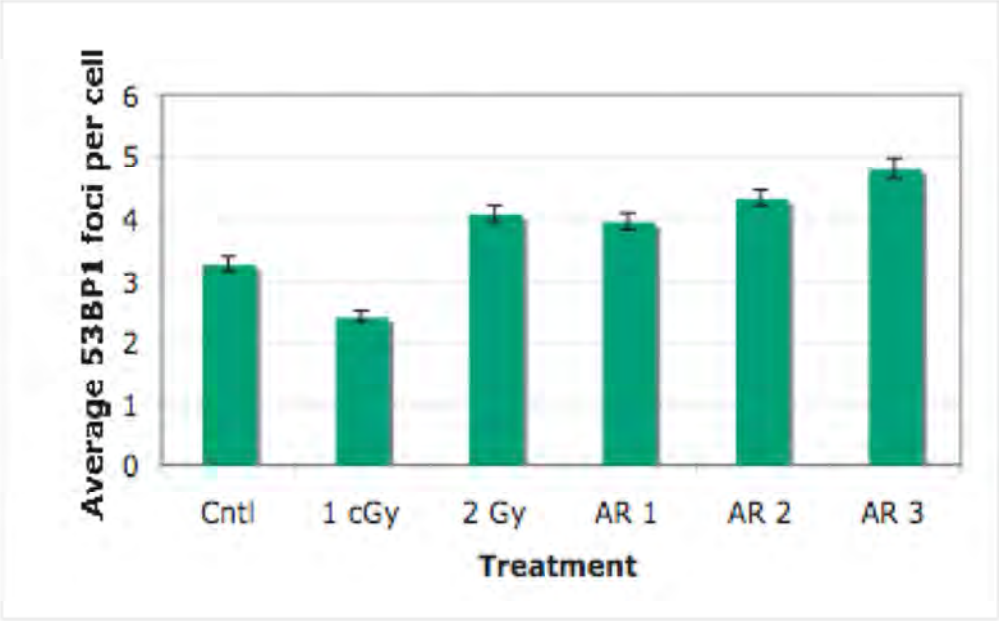
53BP1 foci generation after low and high dose radiation:

184A1 cells were irradiated with 1, 5 and 10 cGy (low dose), 2 Gy (high dose) or nothing (Cntl). After 6 h, foci were detected using immunostaining of 53BP1 and nuclei counterstained with DAPI. Cells were measured for average number of 53BP1 foci. Average numbers were based upon 600-1200 cells counted and standard error was calculated based upon cell number. Duplicate experiments were used for all calculations.



Potential adaptive response and 53BP1 foci generation in 184A1 cells:

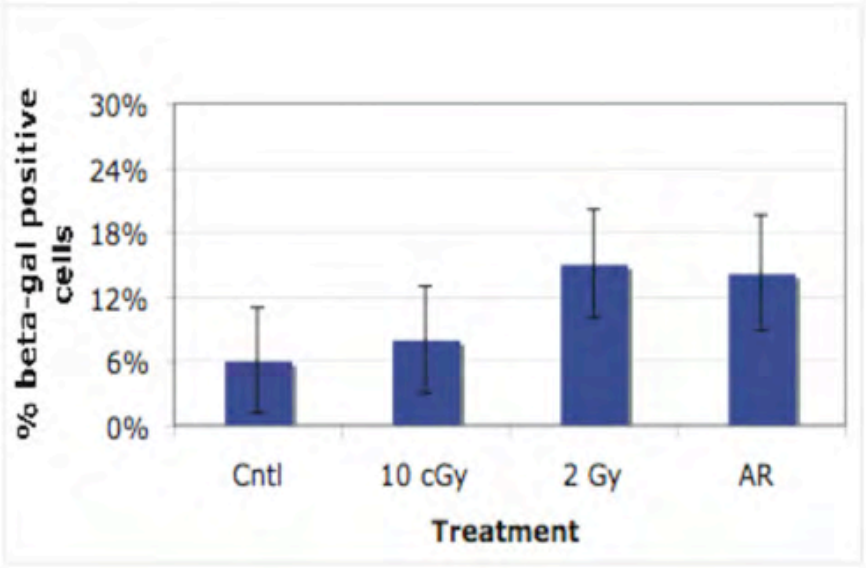
Cells were irradiated with 1 cGy (low dose), 2 Gy (high dose), both or nothing (Cntl). AR1 had an interval of 4 h between doses and cells were collected 6 h after the high dose, AR2 had an interval of 4 h between doses and cells were collected 24 h after the high dose, and AR3 had an interval of 24 h between doses and cells were collected 6 h after the high dose. Cells were immunostained with for 53BP1 and analyzed for average number of foci per cell.



Potential adaptive response and accumulation of SA-β-gal positive 184A1 cells:

A) Cells were irradiated with 10 cGy (low dose), 2 Gy (high dose), both (AR) or nothing (Cntl) and then stained 8 d after ionizing radiation for SA-β-gal. Cells were scored as positive or negative. On average, 200-300 cells were counted per condition and error bars depict duplicate experiments. B) SA-β-galactosidase staining was used to detect senescent cells after ionizing radiation. Positive cells are those that have cytoplasmic dark blue staining. Shown are representative fields.

A



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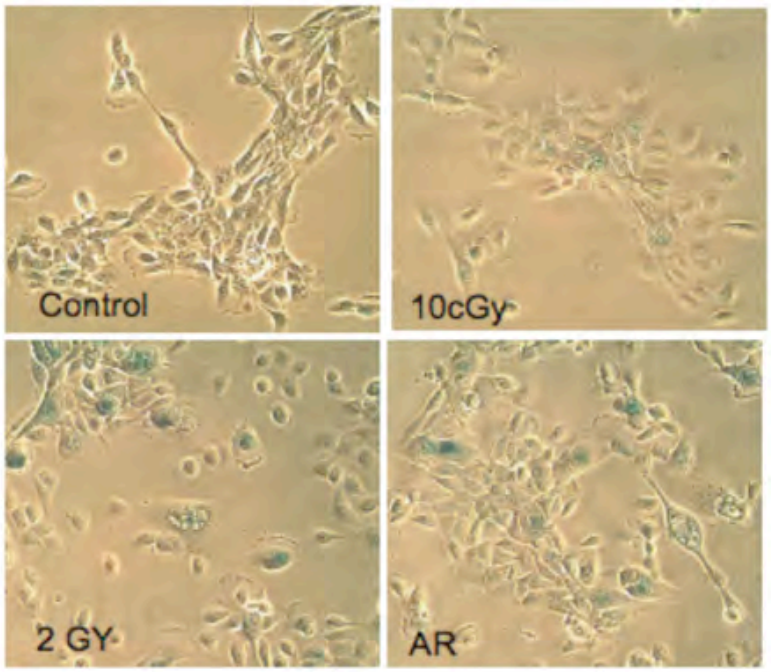
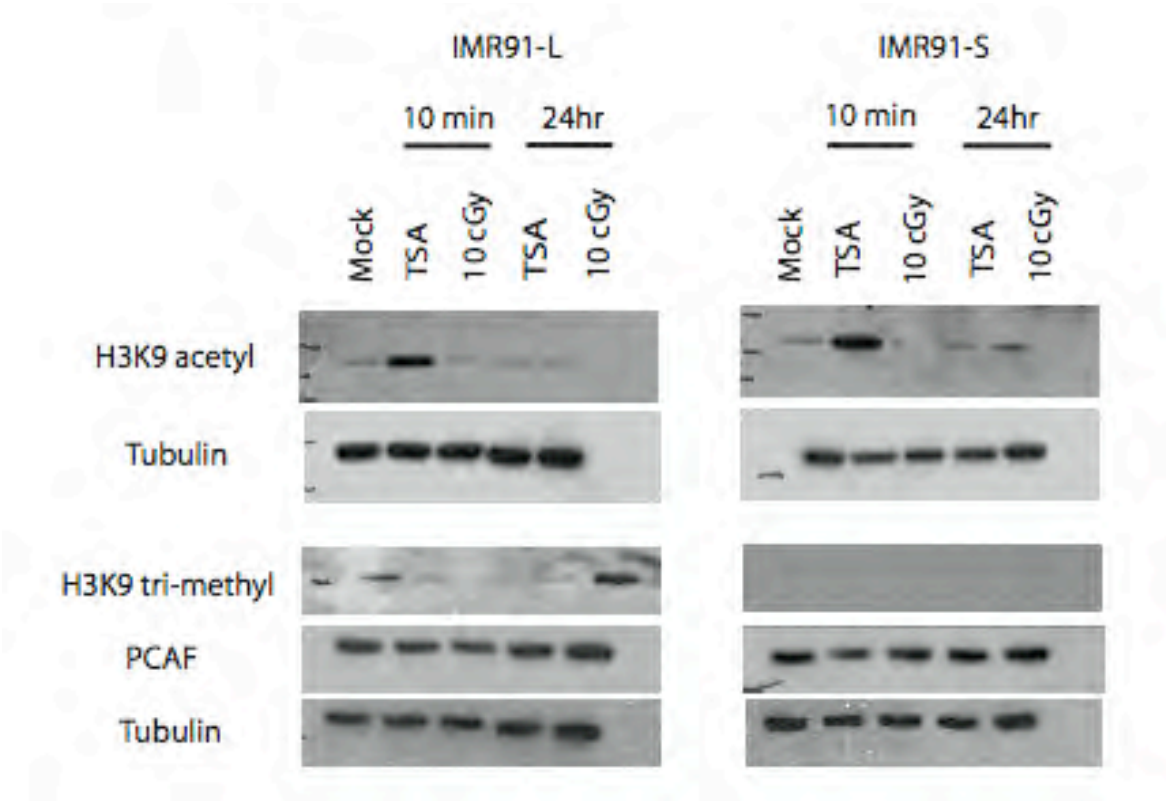


Based on these results, we could conclude that there was no significant change in the amount of DNA damage foci generated (53BP1) or in the induction of senescence (SA-β-gal) when cells were pre-treated with low doses of ionizing radiation and subsequently challenged by a high dose of ionizing radiation. We also investigated the effect of low doses of radiation on chromatin modifications. Our preliminary results indicated that there was no effect on histone methylation or acetylation in mammary epithelial cells.

Even though understanding the effects of low dose radiation in breast epithelial cells was important considering the use of low dose X-rays in the clinic, previous work suggested that human fibroblasts exhibited a radioadaptive response using a variety of endpoints. It was observed that low doses (5-10 cGy) protected normal fibroblasts from the effects of high dose radiation (5 Gy) using the generation of damage foci and induction of senescence. We therefore investigated the potential effects of low doses of radiation on chromatin modifications.

Chromatin modifications in fibroblasts:

Cells were treated with nothing, 10 μM TSA (histone deacetylase inhibitor) or 10 cGy and collected 10 min or 24 h after treatment. Cells were lysed with 5% SDS, centrifuged and protein concentration was determined. Gels were loaded with 20 μg of protein, run and transferred to a PVDF membrane. Membranes were incubated with antibodies against H3K9 tri-methyl, H3K9 acetyl, PCAF or tubulin (which is used as a loading control and whose expression should not be affected by irradiation).



We looked at markers of acetylation and methylation, which indicate euchromatic and heterochromatic conformations after low dose IR. We investigated a common, but specific, histone residue (H3K9) that can undergo modifications through both acetylation and methylation. Upon treatment of human fibroblasts (IMR91-L and IMR91-S) with 10 cGy, there was no significant change in acetylation in any of these two cell populations. In an initial experiment using the IMR91-L cells, there was an increase in H3K9 tri-methylation 24 h after 10 cGy treatment. However, after repeating the experiment multiple times, we could

not consistently confirm this modification. Therefore, at this specific residue, there may be a change upon treatment with low doses, but this modification does not appear to be reproducible.

KEY RESEARCH ACCOMPLISHMENTS

- We cultured human mammary epithelial cells.
- We irradiated these cells using with X-rays at low doses, high doses, or at low doses followed by high doses.
- We measured the effects of these various doses on the number of DNA damage foci.
- We measured the effects on the ability to trigger cell senescence.
- Mammary epithelial cells had no visible adaptive response in terms of a decrease in 53BP1 foci generation or senescence.
- Low doses of radiation had no effect on chromatin modifications in human mammary epithelial cells.
- Low doses of radiation appeared to have an effect on chromatin modifications in human fibroblasts, but which was not consistently obtained.

REPORTABLE OUTCOMES

Based on the data obtained so far, it appears that human fibroblasts, but not human epithelial cells, display a hormetic response when using radiation as a stressor. Since almost all breast tumors originate from epithelial cells, it is unlikely that low doses of radiation could induce a direct protective response against DNA damage and therefore breast cancer. However, the hormetic response of fibroblasts could indirectly impact the initiation and/or progression of breast tumor cells. We are planning to obtain preliminary data in order to answer to this question. We would then submit a grant to fully investigate this hypothesis.

CONCLUSIONS

Many women do not routinely complete screening, particularly women with poor social conditions. Amongst the reasons for the non-compliance, is the fear of radiation. Is this fear justified? Our goal was to obtain some data for or against the idea that low doses of radiation might be beneficial or deleterious. Our data so far suggest that low doses of radiation are not protective, nor detrimental, in mammary epithelial cells (whereas in other cell types, such as fibroblasts, these low doses of radiation appear to be protective). It was important to keep the experiments biologically relevant to women who are exposed to radiation, and this is why we used low doses equivalent to mammography and high doses equivalent to radiation therapy. We cannot rule out that a radioadaptive response occurs in epithelial cells in culture when exposed to different doses of radiation (higher low doses and/or different high doses). However, these conditions would not mimic the doses received during screening and treatment.

REFERENCES

None